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Determination of bioactive properties and quantitative values of phenolic components of different layers of pineapple fruit

Bestimmung der bioaktiven Eigenschaften und der quantitativen Werte der phenolischen Bestandteile verschiedener Schichten der Ananasfrucht

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Summary

The study aimed to assess the differences in bioactive properties and phenolic compounds by HPLC of pineapple cut into pieces such as core, pulp and peel. Total carotenoid content of the pineapple showed a regular increase from the inner to the outer parts. Total phenolic content reached its highest value (70.98 mg GAE/100 g) in the middle part of the pineapple pulp. This part presented similar trends of antioxidant activity by both DPPH and FRAP. The pineapple peel was rich in total flavonoid content (57.67 mg QE/100 g). The main phenolic compounds of the pineapple were catechin (10.70–18.92 mg/100 g), gallic acid (10.18–17.25 mg/100 g) and ferulic acid (1.40–6.10 mg/100 g). The bioactive properties of the fruit showed differences based on the parts of pineapple. These differences were observed not only in the core, pulp and peel parts, but also in the inner or outer parts of the pulp.

Keywords: Bioactive properties, food analyses, HPLC, pineapple parts, PCA, phenolic compounds

Introduction

Pineapple (*Ananas comosus* L.), belonging to the Bromeliaceae family, is one of the fruits with significant commercial value, and cultivated in tropical and subtropical areas such as Southeast Asia and Latin America (Li et al., 2014; Yahya et al., 2019). The pineapple contains about 85% carbohydrates in total solids (13–19%), mostly consisting of sucrose, glucose and fructose (Chaudhary et al., 2019). In addition to carbohydrates, pineapple is an important source of the bioactive compounds such as antioxidants, phenolics, organic acids, bromelain, and can also be used for the production of these value-added compounds (Ali et al., 2020). Especially the pineapple by-products are economical and easily available raw materials from which these compounds are obtained (Tanaka et al., 1999; Kumar et al., 2003; Imandi et al., 2008). The main pineapple by-product is the peel (29–42%), followed by the core (9–20%) and crown (Rico et al., 2020). Several studies carried out by Freitas et al. (2015), Morais et al. (2017) and Banerjee et al. (2018) have shown that not only pineapple pulp but also its by-products are rich in vitamins, minerals, and phenolic compounds. Therefore, half of the weight of the pineapple consists of by-products, which are sources of health-beneficial components (Roda and Lambri, 2019).

Polyphenolic compounds, which are secondary plant metabolites, have health-beneficial properties such as antioxidant, anti-inflammatory and cardioprotective effects (Middleton et al., 2000; Manach et al., 2005; Özcan and Uslu, 2023a,b). It has been reported that the antioxidant activities of polyphenols is related to the number of hydroxyls, and the higher number has the greater antioxidant effect. (Rafat Hussain et al., 1989). Polyphenols exhibit their antioxidant effects in different ways such as free radical scavenging, hydrogen donation, singlet oxygen quenching and metal ion chelation (Haripyaree et al., 2010). Studies continue on polyphenols, which are promising in preventing cancer, cardiovascular diseases and protecting human health by reducing free radical damage (Choi and Lee, 2009). However, there is a lack of publications about bioactive properties of the center, middle or outer parts of the fruit. This study was performed to compare the bioactive properties of pineapple that cut into five parts from the core to the peel.

Materials and methods

Materials

Pineapple (*Ananas comosus* L.) fruits (25 pieces) (Queen variety) were obtained from a local market in Konya, Turkey. The pineapple was divided down the middle and then splitted into layers. The part of 1 was core of the pineapple (diameter is 3.0 cm). The parts of 2, 3 and 4 were divided into parts of equal thickness (1.4 cm) from the inside to the outside (Fig. 1). The 5th part was the peel of the pineapple.

Methods

Moisture content

The water contents of the pineapple samples were detected by the KERN & SOHN GmbH electronic moisture analyser.

Total carotenoid content

Extraction of carotenoids was performed according to Silva da Rocha et al. (2013). The sample (2 g) was added to 25 ml of acetone. The mixture was shaken by vortex for 10 min and filtrated using filter paper (Whatman No. 1), followed by taking in a seperation funnel. The filtrate was fractionated with 20 ml of petroleum ether and washed with 100 ml of distilled water in order to remove the acetone. The washing step was repeated twice. Whatman No. 1 covered with anhydrous sodium sulfate (5 g) for removing residual water was used to filtrate the petroleum ether layer. The volume of the extracts was completed to 25 ml by petroleum ether. After these procedures, the absorbance was measured at 450 nm by spectrofotometer (Shimadzu UV mini 1240, Japan).

Extraction process

The extraction process was performed with some modifications according to a study reported by Hossain and Rahman (2011). The methanol/water solution (80:20, v/v, 20 ml) was added to the pineapple sample (5 g). The mixture was kept in shaking water-bath for 1 h and then filtered through a filter paper (Whatman No. 1). The supernatant was removed, and these steps were repeated twice. The extract collected was evaporated at 40°C in a rotary evaporator under vacuum, followed by dissolved in 10 ml of methanol:water solution (80/20, v/v).

Total phenolic content

Total phenolic contents of the pineapple extracts were carried out using Folin Ciocalteu (FC) reagent (Yoo et al., 2004). The extract (0.5 ml) was mixed with 2.5 ml of FC reagent and 1.5 ml of sodium carbonate solution. The absorbance values of the samples after stored for 2 hours at room temperature in the dark were measured at 725 nm in a spectrofotometer. Gallic acid was used as a standard and the results were expressed as mg gallic acid equivalent (GAE)/100 g.

Total flavonoid content

Total flavonoid contents of the pineapple extracts were detected according to the method recorded by Hogan et al. (2009). The extract (1 ml) was mixed with 0.3 ml of $NaNO_2$, 0.3 ml of $AlCl_3$ and 2 ml of NaOH, respectively. The absorbance of the mixture was recorded at 510 nm with a spectrophotometer. The results were given as mg quercetin equivalent (QE)/100 g.

Antioxidant activity

DPPH free radical scavenging activity

Antioxidant activities of the pineapple extracts were determined using 2,2-diphenyl-1-picrazil (DPPH) as proposed by Lee et al. (1998). After the extract (0.1 ml) was mixed with 2 ml of DPPH solution, the absorbance value of the sample, which was kept in the dark for 30 min at room temperature, was recorded at 517 nm in a spectrofotometer. The results were given as mmol trolox equivalent (TE)/kg.

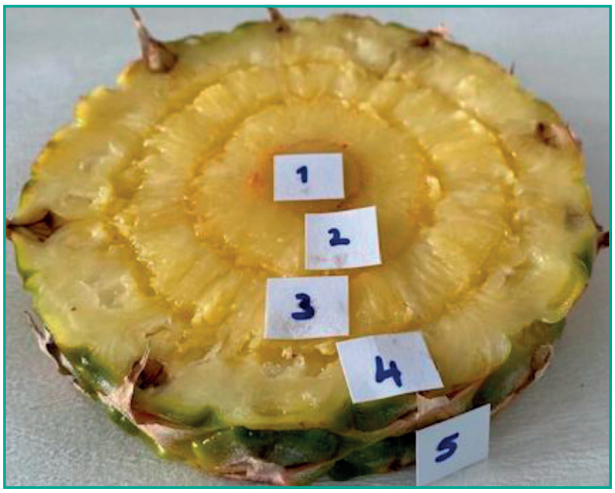

FIGURE 1: *Parts of pineapple.*

Journal of Food Safety and Food Quality 75, Heft 3 (2024), Seiten 65–92

Ferric reducing antioxidant power (FRAP)

Antioxidant activities of the pineapple extracts were found using FRAP reagent reported by Mudenuti et al. (2021). The FRAP reagent was constituted of 25 mL of 0.3 mM acetate buffer (pH 3.6), 2.5 mL of 10 mM 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ), and 2.5 mL of 20 mM iron (III) chloride. The sample (10 µl) was mixed with 900 mL of FRAP reagent, and the mixture was kept in the dark at room temperature for 30 min. The absorbance at 595 nm was read using a spectrophotometer. The results were expressed as mg $FeSO_4$/100 g.

Determination of phenolic compounds

The phenolic compounds of the pineapple extracts were quantified at 280 nm by HPLC (Shimadzu LC 10A vp, Kyoto, Japan) equipped with Inertsil ODS3 analytical column (5 µm, 25 cm x 4.6 mm, GL Sciences, Japan) and a Diode Array Detector (Shimadzu SPD-M20). Phenolic compounds were separated by gradient elution method using mobile phases of A (0.5% acetic acid aqueous solution), and B (acetonitrile). The gradient program was as follows: 0–0.10 min 8% B; 0.10–2 min 10% B; 2–27 min 30% B; 27–37 min 56% B; 37–37.10 min 8% B; 37.10–45 min 8% B. The flow rate of the mobile phases was 0.85 ml/min, and the injection volume was 20 µl. The column temperature was maintained at 40°C during the run. Reference standards for phenolic compounds were obtained from Sigma-Aldrich Co. LLC.

Statistical analyses

The statistical analyses of the results were carried out using SPSS-Statistics-22 statistical program and data were analysed using one-way ANOVA. The means of significant variation sources were compared using Duncan Multiple Comparison Test with the help of MSTAT program. The significance level was given as $p < 0.05$ unless stated otherwise. The analyses were repeated 3 times (n = 3). The principal component analysis (PCA) was applied using JMP Pro 16.

Results and discussion

Bioactive compounds and antioxidant activity values of different parts of the pineapple

Water contents and bioactive properties of different parts of the pineapple are presented in Table 1. The water contents of the pineapple parts varied between 85.53% and 89.79%. The core of the sample had the highest water content (89.79%), followed by 4th part (88.52). Carotenoid contents of the pineapple parts showed a steady increase from inner side (0.27 µg/g) to outer piece (5.76 µg/g). It was seen that the peel of the pineapple (5th part) was rich for carotenoid content. The values were in accordance with the results recorded by Steingass et al. (2020), who found total carotenoid contents of the ripe pineapple pulps with the range of 29–565 µg/100 g. In another study, total carotenoid amounts of different parts of the pineapple wastes were equal to 0.35 µg/g (dw) for core; 0.49 µg/g (dw) for pomace; 1.82 µg/g (dw) for peel (Sengar et al., 2022). It was informed that the external pericarp and peel had higher total carotenoid content than inner parts of the fruit (Martins and Ferreira, 2017). Accordingly, similar to current study, the peels of pineapple, banana and avocado contained 3.7, 5.3 and 2.6 times higher carotenoid amounts in comparison to their pulps, respectively (Ayala-Zavala et al., 2011).

Total phenolic contents of samples ranged from 46.02 mg/100 g to 70.98 mg/100 g. These values were closed to the previously reported total phenolic contents (31.48 and 77.55 mg GAE/100 g fw) in 26 pineapple genotypes from China (Lu et al., 2014). To compare the effect of the pineapple parts on total phenolic content, the highest and the lowest values were observed in middle part (3rd) and inner part (1st) of the pineapple, respectively. Similarly, in another study, the results revealed that total phenolic contents were in the descending order of pulp (26.29 mg GAE/g dw), peel (25.91 mg GAE/g dw) and core (14.12 mg GAE/g dw) (Sengar et al., 2022). In previous studies, the total phenolic contents were recorded as 148.91 mg GAE/100 g fresh weight (fw) in the pineapple peel obtained by Li et al. (2014); 21.7 mg GAE/100 g in the pineapple pulp reported by Kuskoski et al. (2006), which were respective higher and lower than current study. In several studies carried out by Li et al. (2014), Hossain et al. (2011) and de Oliveira et al. (2009), the higher total phenolic contents were presented as 31.98 mg GAE/g, 51.1 mg (CAE)/g and 9.1 mg GAE/g extract of the pineapple. On the other hand, lower values compared to the current study were dedected in pulp of Phulae pineapple (26.20 mg GAE/100 g) and Nanglae pineapple (20.28 mg GAE/100 g fw) recorded by Kongsuwan et al. (2009). While the total phenolic quantities of different parts of Pineapple were significantly higher than the study results of Sengar et al., (2022), Kongsuwan et al. (2009) and Oliveira et al. (2009), total phenolic quantity of pineapple fruits were assessed lower than that of result of Li et al. (2014. The possible reasons of differences could be the different variety, extraction process, location and climate conditions.

Total flavonoid contents of the pineapple parts were equal to 12.71–57.67 mg/100 g. This finding was in accordance with the study of Fidrianny et al. (2018), who found total flavonoid content in the value of 0.0345 g RE/100 g (fw) in Comte de Paris pineapple pulp. However, Fidrianny et al. (2018) presented higher flavonoid amount in Bogor pineapple both pulp (0.73 g QE/100 g dw) and peel (0.17 g QE/100 g dw). In general, an increase was detected from the core to the peel of pineapple, which was similar to carotenoid amounts. Similar to present study, the highest flavonoid content was observed in the peel of pineapple (8.15 mg QE/g dw), followed by pomace (3.47 mg QE/g dw) and core (2.23 mg QE/g dw) (Sengar et al., 2022). In addition, Alothman

TABLE 1: *Water content and bioactive properties of the pineapple parts.*

Part of pineapple	Water content (%)	Carotenoid content (µg/g)	Total phenolic content (mg/100g)	Total flavonoid content (mg/100g)	Antioxidant activity (DPPH, mmol/kg)	Antioxidant activity (FRAP, mg/100g)
1	89.79 ± 0.95*^{A}	0.27 ± 0.03^{E}	46.02 ± 1.23^{D}	12.71 ± 0.29^{D}	1.52 ± 0.00^{C}	315.80 ± 3.63^{C}
2	85.82 ± 0.07B**	1.52 ± 0.03^{D}	61.64 ± 0.57^{C}	21.00 ± 0.76^{C}	1.54 ± 0.00^{B}	455.60 ± 5.78^{B}
3	86.08 ± 0.76^{B}	1.78 ± 0.00^{C}	70.98 ± 1.81^{A}	20.71 ± 0.57^{CD}	1.56 ± 0.01^{A}	544.00 ± 5.88^{A}
4	88.52 ± 0.32^{AB}	2.44 ± 0.03^{B}	66.02 ± 0.83^{B}	30.05 ± 0.59^{B}	1.53 ± 0.00^{C}	461.30 ± 5.60^{B}
5	85.53 ± 0.21^{B}	5.76 ± 0.03^{A}	61.98 ± 1.30^{C}	57.67 ± 0.87^{A}	1.44 ± 0.00^{D}	309.80 ± 4.38^{C}

*mean (three replicates) ± standard deviation of each parameter. **Different superscript letters in the same column indicate significant differences ($p<0.01$) were compared with Duncan test.

et al. (2009) reported that total flavonoid contents of fresh fruit extracts dedected as 3.70 mg CAE/100 g extracted with methanol: water (50:50). Opposite to that Adeboyejo et al. (2018) reported higher total flavonoid amounts varied between 0.51 and 1.89 mg/g. According to the study of Hossain and Rahman (2011), the main reason of the variation could be the environmental conditions, which are effective on constituents of the plant. The total flavonoid contents of different parts of Pineapple were significantly higher than the study results of Fidrianny et al (2018), Sengar et al (2022), Alothman et al (2009) and Adeboyejo et al (2018).

Antioxidant activities of the pineapple samples were determined as 1.44–1.56 mmol/kg by DPPH method; 309.80-544.00 mg/100 g by FRAP method. The pineapple extracts exhibited similar trends of activity in both the DPPH and FRAP radical scavenging methods. The middle of the fruit (part of 3), which behaved similar to total phenolic contents of samples, presented the highest antioxidant activity for both methods. Similarly, the highest total antioxidant capacity was observed in the pulp of pineapple (Sengar et al., 2022). However, there was a decreasing tendency in the antioxidant activity towards the peel of sample. In an experiment carried out by Kongsuwan et al. (2009), the antioxidant activity by DPPH and FRAP was dedected as 152.93 mol TE/100 g (fw) and 205.73 mol ME/100 g (fw) in the frozen pulp of Nanglae pineapple; 118 mol TE/100 g (fw) and 165.28 mol ME/100 g (fw) in Phulae pineapple. Previous study of Lu et al. (2014) presented that DPPH scavenging activities of the pineapple pulps varied between 3.68 and 22.85 µmol TE/g (fw) in 26 genotypes of pineapples from China, which values of some genotypes such as Giant Kew and CPM were closed to current study. In another study, the ethanolic extracts of pulp and peel of Bogor pineapple exhibited 375.58 and 259.08 µg/ml of EC50 using FRAP method, respectively (Fidrianny et al., 2018). By using the DPPH method, the inhibitions of ethanolic extracts of the pineapple cultivars were presented between 7.09% and 13.38% by Adeboyejo et al. (2018). The phenolic compounds have drawn attention thanks to the antioxidant activity, which increases with increasing hydroxylation degree (Li et al., 2014). Additionally, phenolic compounds contained ortho- and para-dihydroxylation exhibite higher antioxidant activity than simple phenolics (Shahidi & Naczk, 1995).

Phenolic compounds of different parts of the pineapple

Phenolic compounds of the pineapple parts are displayed in Table 2. The amounts of gallic acid, catechin, caffeic acid, p-coumaric acid and ferulic acid showed significant differences ($p < 0.01$) according to the parts of fruit; in contrast, the quantity of 3,4-dihydroxybenzoic acid, syringic acid, rutin, quercetin, cinnamic acid and kaempferol were not significantly affected by the different parts of pineapple ($p > 0.05$). Catechin (10.70-18.92 mg/100 g) was the main flavonoid of the pineapple, and the parts of 3-5 contained the highest level of this compound. The major phenolic acid of the pineapple was gallic acid (10.18-17.25 mg/100 g), and its maximum value was observed in 2nd part of fruit. The results revealed that ferulic acid contents of the pineapple decreased from 6.10 mg/100 g to 1.40 mg/100 g as progressed from the inner part to the outer part. Moreover, the highest caffeic acid (0.43 mg/100 g) and p-coumaric acid (0.21 mg/100 g) amounts were determined in the peel of fruit. Similarly, gallic and ferulic acids were identified as the main phenolic acids of pineapple waste in several studies reported by Sopie et al. (2011), Sepulveda et al. (2018), Campos et al. (2020) and Polania et al. (2022). Selani et al. (2016) informed that the mixture of peel and pulp of the pineapple contained ferulic, caffeic and p-coumaric acids with the values of 5.1 mg/100 g, 2.9 mg/100 g and 1.71 mg/100 g, respectively. In another study carried out by Li et al. (2014), gallic acid (31.76 mg/100g dw), catechin (58.51 mg/100g dw) and ferulic acid (19.50 mg/100g dw) were observed in the pineapple peels, which were higher than current study due to probably calculation the results in dry or fresh weight. Ferulic acid contents after the first layer of pine apple fruit were lower than the ferulic acid results of Selani et al (2016). From our findings, gallic and catechin acid contents were found to be lower than the results of Li et al (2014). These differences may possibly be due to the variety, genetic structure, growing conditions, and different parts of the pineapple.

Multivariate statistical analysis

Principal component analysis (PCA) was performed to determine the differences in bioactive properties and parts of pineapple. Figure 2 gives biplot graph which was drawn using the first component (PC1) and the second component (PC2). PC1 and PC2 explained 52.8% and 27% of total va-

TABLE 2: *Phenolic compounds of the pineapple parts.*

Part of pineapple	Gallic acid (mg/100 g)	3,4-Dihydroxybenzoic acid (mg/100 g)	Catechin (mg/100 g)	Caffeic acid (mg/100 g)	Syringic acid (mg/100 g)	Rutin (mg/100 g)
1	$11.29 \pm 0.50^{*B}$	$1.30 \pm 1.09^{**}$	$10.70 \pm 0.51^{B***}$	0.06 ± 0.02^{C}	0.07 ± 0.02	1.70 ± 0.22
2	17.25 ± 1.22^{A}	1.23 ± 0.33	10.76 ± 0.64^{B}	0.23 ± 0.12^{B}	0.20 ± 0.10	2.54 ± 0.64
3	12.24 ± 2.62^{B}	0.65 ± 0.14	18.92 ± 0.53^{A}	0.10 ± 0.04^{BC}	0.29 ± 0.25	2.62 ± 0.38
4	10.98 ± 0.25^{B}	0.76 ± 0.38	16.97 ± 1.60^{A}	0.08 ± 0.01^{BC}	0.08 ± 0.02	2.43 ± 0.02
5	10.18 ± 0.15^{B}	0.70 ± 0.77	17.36 ± 2.58^{A}	0.43 ± 0.05^{A}	0.55 ± 0.64	1.16 ± 0.84

Part of pineapple	*p*-coumaric acid (mg/100 g)	Ferulic acid (mg/100 g)	Quercetin (mg/100 g)	Cinnamic acid (mg/100 g)	Kaempferol (mg/100 g)
1	0.08 ± 0.01^{AB}	6.10 ± 0.05^{A}	0.07 ± 0.04	0.01 ± 0.00	0.04 ± 0.01
2	0.03 ± 0.00^{B}	5.28 ± 0.09^{A}	0.29 ± 0.07	0.04 ± 0.00	0.08 ± 0.01
3	0.02 ± 0.00^{B}	4.79 ± 0.01^{A}	0.19 ± 0.10	0.06 ± 0.00	0.04 ± 0.00
4	0.07 ± 0.00^{B}	3.05 ± 0.07^{B}	0.12 ± 0.10	0.01 ± 0.01	0.07 ± 0.01
5	0.21 ± 0.01^{A}	1.40 ± 0.07^{C}	0.18 ± 0.20	0.04 ± 0.01	0.14 ± 0.04

*mean (three replicates) ± standard deviation of each parameter. Different superscript letters in the same column are not significant (**p>0.05). Different superscript letters in the same column indicate significant differences (***p<0.01) were compared with Duncan test

Journal of Food Safety and Food Quality 75, Heft 3 (2024), Seiten 65–92

riance, respectively (Table 3). PC1 demonstrated a strong positive correlation with total flavonoid, total carotenoid, p-coumaric acid, kaempferol, caffeic acid and syringic acid contents, and also the P5 (peel of pineapple) contained the highest levels of these compounds. In addition, a higher positive correlation was observed in total phenolic content and antioxidant activity by FRAP with PC2. The P3 (3rd part of pineapple) had a remarkable antioxidant activity (FRAP) and total phenolic content in comparison to other parts. The P1 (core of the pineapple) was located in the negative area of both PC1 and PC2, and the maximum amounts of ferulic and 3,4-dihydroxybenzoic acids were reached in P1, which were located in the same area. It can be seen that, in general, the bioactive properties of pineapple show differences from inner to outer part.

Conclusions

This study highlighted the significance of pineapple parts (core, pulp-inner, middle and outer parts- and peel) on total carotenoids, phenolics, flavonoids, antioxidant activity and phenolic compounds. By using the PCA, it could be seen that there were major differences among core, pulp and peel; minor changes in the parts of pulp. It had been determined that the peel (part of 5) can be preferred for total carotenoid and flavonoid amounts, and the middle part (part of 3) is rich in total phenolic content and antioxidant activity. On the base of the phenolic compounds, the highest amount of gallic acid was found in the 2nd part, while the amount of ferulic acid was the maximum in the 1st part and decreased as it progressed to the peel. Moreover, catechin amounts in 3–5 parts of fruit were dedected at high levels. From the results obtained, it could be concluded that we can prefer inner part of pulp for gallic acid; outer part of pulp for catechin; peel for caffeic and p-coumaric acids; core for ferulic acid.

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Conflicts of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethical statement

This is to inform you that in this study, we have not been involved in any animal and human studies.

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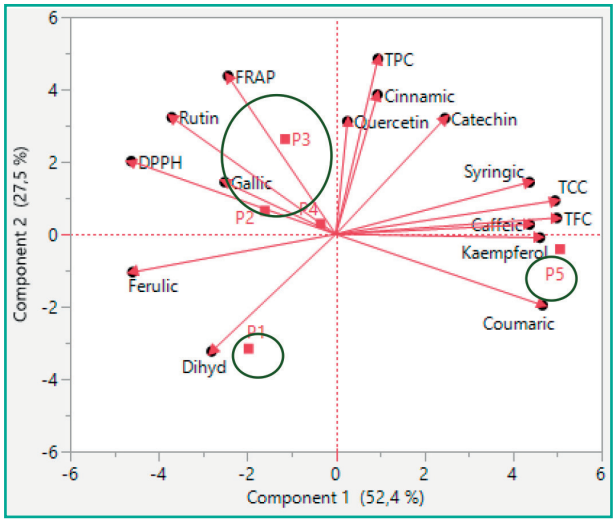

FIGURE 2: *Biplot graph drawn with results of PCA for pineapple.*

TABLE 3: *PCA results in relation to bioactive properties of pineapple.*

	PC1	PC2	PC3	PC4
Eigenvalue	8.38	4.40	2.50	0.72
Variability (%)	52.36	27.47	15.64	4.52
Cumulative %	52.36	79.83	95.48	100.00
Correlation				
TCC	0.977	0.183	0.016	−0.103
TPC	0.187	0.957	−0.152	−0.160
TFC	0.985	0.089	0.011	−0.145
DPPH	−0.912	0.399	−0.094	0.002
FRAP	−0.481	0.864	−0.126	−0.085
Gallic	−0.495	0.285	0.804	−0.167
Dihyd	−0.554	−0.639	0.533	−0.028
Catechin	0.487	0.633	−0.595	0.093
Caffeic	0.864	0.053	0.500	0.020
Syringic	0.864	0.284	0.237	0.343
Rutin	−0.732	0.638	−0.012	−0.240
Coumaric	0.922	−0.387	−0.029	0.017
Ferulic	−0.907	−0.207	0.201	0.309
Quercetin	0.052	0.616	0.781	−0.091
Cinnamic	0.186	0.762	0.322	0.530
Kaempferol	0.910	−0.018	0.331	−0.249

TCC: Total carotenoid content, TPC: Total phenolic content, TFC: Total flavonoid content, DPPH: Antioxidant activity using DPPH method, FRAP: Antioxidant activity using FRAP method, Dihyd: 3,4-Dihydroxybenzoic acid.

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